The method of Claim 19 wherein the mixed intracellular and extracellular [removed] proteins have been precipitated together from a combined mixture of the first and second supernatants using acetone and then isolated and dispensed in sterile distilled water to provide the vaccine.

**REMARKS**

Claims 16 to 25 are pending. Claims 26 and 27 are canceled. No claims are allowed. The present amendment repeats in part the assertions of the earlier Amendment Under 37 CFR 1.116(a) which was not entered.

Enclosed is a Declaration Under 37 CFR 1.132 by the inventor. As can be seen, the assertions in the Advisory Action dated February 17, 2000 are incorrect as to the meaning of "extracellular antigen".

Claims 19, 24 and 25 have been corrected as required. Claim 18 as amended cannot crate any "new matter", since it duplicates cancelled Claim 27.

Independent Claims 16 and 18 were amended to limit the claims to the specific vaccine that produced the unexpected results. In particular, the amendments limit the claims to a vaccine that consists of mixed intracellular and extracellular proteins which have a molecular weight greater than 10,000. This limitation

was the subject matter of canceled Claims 26 and 27. Support for the limitation can be found in step 10 of the method for making the vaccine wherein the mixed proteins are dialyzed against a membrane with a 10,000 MW cut-off (specification: page 7, lines 34-35). This step removes material of a molecular weight less than 10,000. No further examination is thus required because of this amendment.

1. Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being anticipated by Mendoza *et al.* (IDS: AI; Mycopathologia 119: 89-93 (1992)).

In the present invention, the extracellular antigens are separated from the cell mass and expressed into the culture medium. The extracellular antigens are not part of the outside of the cells as seems to be implied by the Office Action. The intracellular antigens are all of the antigens of the cell including any surface antigens. The pending claims are clear as to the distinction between the extracellular antigens and the intracellular antigens. Furthermore, the vaccine is dialyzed to remove low molecular weight materials. There is no suggestion in any of the prior art to take this step. It is these vaccines which were tested in the horses and found to provide the unexpected efficacy in the nature of a synergism.

The present invention provides a vaccine that consists of an admixture of soluble extracellular antigens from the growth medium and soluble intracellular antigens from disruption of the cell mass. In contrast, Mendoza (AI) teaches two vaccines, a soluble concentrated antigen vaccine (SCAV) consisting solely of extracellular antigens that are extruded by the cell into the medium, and a cell-mass vaccine (CMV) consisting solely of intracellular antigens from disruption of the cell mass and cell debris. Both prior art vaccines are distinguishable from the vaccine of the present invention.

The SCAV disclosed in Mendoza (AI) was prepared according to Mendoza and Alfaro in Mycopathologia 94: 123-129 (1986) by removing the cells from the medium and then isolating from the medium those antigens that had been extruded by the cells into the medium, i.e., the extracellular antigens. Since the cells were removed from the medium intact, and not disrupted to release intracellular antigens, the SCAV does not contain intracellular antigens. The method disclosed in Mendoza (AI) omitted describing the step of filtering the medium to remove the cells from the medium before concentrating the medium 20-fold in a stir cell (Mendoza (AI): sentence spanning pages 90-91). However, note that Mendoza (AI) cites that it followed the method

of Mendoza and Alfaro (*ibid.*) which describes the omitted step. In contrast to the SCAV, the vaccine of the present invention contains both soluble extracellular extruded into the medium and all of the soluble intracellular antigens (specification: page 7, steps 3-6). Therefore, the vaccine of the present invention which contains both soluble intracellular and soluble extracellular antigens is distinguishable from the SCAV which consists solely of extracellular antigens.

The CMV of Mendoza (AI) was prepared from a cell mass that had been washed free of medium containing extracellular antigens. The cell mass was then disrupted to expose the intracellular antigens. After disruption, the cell mass was desiccated overnight to produce a dried composition consisting of intracellular antigens and cell debris which was then dissolved in saline to produce the vaccine. Thus, the CMV consists solely of intracellular antigens and cell debris; it does not contain the extracellular antigens that were extruded into the medium. In contrast to the CMV, the vaccine of the present invention consists of an admixture of soluble extracellular antigens extruded into the medium and the soluble intracellular antigens released from the cells after disruption. The vaccine of the present invention does not contain the insoluble

cell debris (specification: page 7, steps 4-6). Thus, the vaccine of the present invention is distinguishable from the CMV vaccine of Mendoza (AI).

Since neither the CMV nor the SCAV of Mendoza (AI) contains both the soluble extracellular and the soluble intracellular antigens, the CMV and SCAV do not anticipate the present invention or render it obvious to one skilled in the art under 35 U.S.C. § 103(a). Therefore, reconsideration of the rejection is requested.

2. Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being unpatentable over Mendoza *et al.* (J. Mycol. Med. 6: 151-164 (1996)).

Mendoza *et al.* (1996) discloses a vaccine that contains the 28K, 30K, and 32K cytoplasmic antigens added to the "original *Pythium*-vaccine" (page 159, column 2, lines 15-18). The "original *Pythium*-vaccine" is equivalent to the SCAV of Mendoza (AI). While the Mendoza *et al.* (1996) vaccine contains the 28K, 30K, and 32K intracellular antigens added to the SCAV, not included in the Mendoza *et al.* (1996) vaccine are the many other soluble intracellular antigens which are included in the vaccine of the present invention because of the disruption of the whole cell mass. Enclosed is an abstract dated September 1995 which shows that the

SCAV was mixed with only the specific isolated intracellular antigens. To prepare a vaccine in this manner is too expensive. One skilled in the art could not predict the results from the claimed composition which includes all of the soluble cell mass antigens with the SCAV. Particularly, the dialyzed vaccine as now claimed. Therefore, the vaccine disclosed in Mendoza *et al.* (1996) is distinguishable from the vaccine of the present invention. Thus, Mendoza *et al.* (1996) does not anticipate the present invention. Accordingly, reconsideration of the rejection is requested.

3. Claims 23 and 26-27 were rejected under 35 U.S.C. § 103(a) as being unpatentable over either Mendoza *et al.* (IDS: AI) or Mendoza *et al.* (J. Mycol. Med. 6: 151-164 (1996)) in view of Mendoza *et al.* (IDS: AJ; J. Clin. Microbiol., Nov. 1992, pp 2980-2983) and Panella *et al.* (Cancer Res. 50: 4429-4435 (1990)).

Mendoza (AI) teaches two different methods for producing *Pythium insidiosum* vaccines, (1) a cell-mass vaccine (CMV) containing both the intracellular antigens from disrupted *P. insidiosum* and *P. insidiosum* cell debris, and (2) a soluble concentrated antigen vaccine (SCAV) containing only the extracellular antigens that are extruded by *P. insidiosum* into the cell culture

medium. Both vaccines are effective as immunotherapy vaccines for curing horses infected with *P. insidiosum* for less than 0.5 months (Mendoza (AI): page 92, Table 1). However, these vaccines are of limited efficacy for curing horses infected for greater than 0.5 months but less than 2 months, and neither vaccine is effective for treating horses that had been infected for more than 2 months (Mendoza (AI): page 92, Table 1, and page 93, Tables 3 and 4). Thus, the CMV and SCAV are of similar efficacy. However, Mendoza (AI) teaches that the SCAV is more practical than the CMV because it retains its effectiveness for up to a year after preparation and has a less violent inflammatory reaction at the site of injection than the CMV (page 94, last paragraph). Thus, Mendoza (AI) concludes that the SCAV can be used as the vaccine of choice in early cases of infection (abstract: page 89, last sentence). Therefore, Mendoza (AI) teaches that the CMV and SCAV are equivalent in efficacy but that the SCAV vaccine is preferred because of its longer shelf-life and its lower inflammatory reaction. Finally, Mendoza (AI) further recommends that the components of the SCAV responsible for immunity be determined (sentence spanning pages 92-93). This implies that the preferred vaccine should contain only those extracellular antigens of the SCAV which are responsible for immunity. Thus, Mendoza (AI) leads one

skilled in the art away from the vaccine of the present invention and towards a vaccine that consists of one or more extracellular antigens.

Mendoza (AJ) teaches that intracellular preparations of *P. insidiosum* contain at least 20 antigens which are recognized by antisera from infected horses, and that three of these antigens appear to be immunodominant. Mendoza (AJ) does not teach a vaccine containing the immunodominant antigens but suggests that the three immunodominant antigens may be candidates for vaccination trials. To evaluate these antigens in vaccination trials, it is reasonable to assume that one skilled in the art would prepare vaccines that contained various combinations of the above antigens in isolated form. Thus, Mendoza (AJ) leads one skilled in the art towards vaccines that consists of one or more of the immunodominant antigens isolated from the cell.

Mendoza *et al.* (1996) is a review article about *Pythiosis*. It is not enabling for *Pythiosis* vaccines since it does not disclose methods for making or using *Pythiosis* vaccines. Mendoza *et al.* (1996) states that adding the 28K, 30K and 32K immunodominant antigens to the "original *Pythium*-vaccine" enhanced its curative properties (page 159, column 2, lines 15-18). Adding the 28K, 30K, and 32K intracellular antigens to the SCAV of Mendoza (AI) would not produce the vaccine

of the present invention because the SCAV would not contain the other intracellular antigens. Furthermore, it would be prohibitively expensive to produce a vaccine that contained particular antigens that had to be gel purified. Thus, Mendoza *et al.* (1996) teaches vaccines that contain extracellular antigens supplemented with particular intracellular antigens. There is nothing in Mendoza *et al.* (1996) which would suggest a vaccine that contained all of the intracellular antigens. Therefore, when viewed together, Mendoza (AI), Mendoza (AJ), and Mendoza *et al.* (1996) suggest to one skilled in the art that the preferred vaccine should consist of an admixture of defined intracellular and extracellular antigens.

Panella *et al.* teaches that thimersol induces terminal differentiation in leukemic blasts and accelerated differentiation in normal bone marrow erythroid cultures. Panella *et al.* does not disclose methods for making and using *Pythiosis* vaccines. It is well known that thimersol is a relatively nontoxic antimicrobial preservative (paragraph spanning pages 4433-4434). Panella *et al.* discloses that thimersol is relatively nontoxic; it has a $LD_{50}$ in mice of 120 mg per kg body weight and that up to 2 grams has been given to humans without "toxic effects." Thimersol is used by the applicant to kill *Pythium insidiosum* cells prior to

processing the cells for vaccines. In the present invention, the amount of thimersol used to kill the cells was about 0.2 mg per ml of culture which is considerably less than the $LD_{50}$ for mice. Thus, toxicity of thimersol would not have been a motivating factor for one skilled in the art to dialyze the vaccine (specification: page 7, lines 34-35. Furthermore, the differentiation effect observed in Panella *et al.* was in blasts from patients with leukemia and several human leukemia cell lines (Panella *et al.*: abstract). It is not believed that one skilled in the art would be concerned about thimersol's effect on aplastic anemia in making a *Pythiosis* vaccine. In addition, it is believed that one skilled in the art would know that the precipitation step which precedes the dialysis step (specification: page 7, lines 28-31) would remove a considerable amount of the thimersol. The dialysis step is to remove low molecular weight proteins, salts and sugars used to prepare the medium, and any remaining thimersol. Therefore, the teachings in Panella *et al.* would not have been a motivating influence on one skilled in the art interested in a *Pythiosis* vaccine.

In contrast to the above prior art vaccines, the vaccine of the present invention is more efficacious than either the CMV or SCAV alone. Unexpectedly, the vaccine of the present invention is able to cure horses

that have been chronically infected with *P. insidiosum* for greater than 60 days (Specification: page 8, lines 22-27). The vaccine of the present invention also cures all horses that are acutely infected with *P. insidiosum* (Specification: page 8, lines 32-33). Furthermore, the vaccine of the present invention cured a human who had been infected with *P. insidiosum* for over 60 days. These unexpected and remarkable properties of the vaccine of the present invention are in distinct contrast to the CMV and SCAV of the prior art which are only effective against *P. insidiosum* in horses infected for less than 15 days and marginally effective in horses infected for more than 45 days.

The vaccine of the present invention is not a merely an admixture of CMV and SCAV prepared according to the prior art. The vaccine of the present invention is an admixture of the isolated soluble intracellular antigens separated from the cell mass and the isolated extracellular antigens, wherein components in the admixture less than 10,000 MW have been removed by dialysis. Therefore, the vaccine of the present invention consists of all of the soluble intracellular antigens but without the cell debris that is present in CMV. It would have been unexpected that removing the intracellular antigens associated with the cell debris from a vaccine would enhance the vaccine's efficacy.

Therefore, in view of the unexpected and remarkable properties of the vaccine of the present invention, it would not have been obvious to one skilled in the art that an admixture of soluble intracellular and extracellular antigens would produce a vaccine with properties not evident in vaccines consisting of either the intracellular antigens (CMV) or the extracellular antigens (SCAV) alone.

As stated in *In re Vaeck*, 20 USPQ2d 1438, at 1442 (Fed. Cir 1991),

> [w]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art they should make the claimed composition . . . ; and (2) whether the prior art would also have revealed that in so making . . . , those of ordinary skill would have a reasonable expectation of success . . . .

In light of *In re Vaeck*, the prior art references do not suggest nor imply that the vaccine of the present invention would provide a more efficacious vaccine. Since Mendoza (AI) teaches that neither the CMV consisting of intracellular antigens nor the SCAV consisting of extracellular antigens are able to cure infected horses after 60 days or more of infection, there is no reason for one skilled in the art to believe that an admixture of intracellular and extracellular

antigens would produce a vaccine that would cure infected horses 60 days or more after infection. In particular, the inability of either prior art vaccine to cure infected horses after 60 days or more of infection and the preference of Mendoza (AI) for the SCAV consisting of extracellular antigens teaches away from the present invention. Mendoza *et al.* (1996) teaches that a modified vaccine containing the 28K, 30K, 32K intracellular antigens had enhanced curative properties which implies that the intracellular antigens to be used in a vaccine should be isolated from the cells, not that an undefined mixture of intracellular antigens be used to make the vaccine. Finally, Mendoza (AI) teaches that the immunogenic antigens in the SCAV be identified, ostensively for vaccine use (Mendoza (AI): sentence spanning pages 92-93). Therefore, it would not be reasonable to expect one skilled in the art to combine the total soluble intracellular antigens and total soluble extracellular antigens to make the vaccine of the present invention absent some teaching in the prior art that would suggest doing so would produce a more efficacious vaccine. Furthermore, in view of the prior art, one skilled in the art would have little motivation to produce the vaccine of the present invention because to do so would entail increased effort to produce a vaccine with an expected efficacy no better than the

efficacy of either prior art vaccine. Finally, Panella *et al.* which discloses thimersol can induce leukemic cells to differentiate is not expected to have had any motivating influence on one skilled in the art in preparing a *Pythiosis* vaccine.

Also, as held in *In re Vaeck*, one skilled in the art should have a reasonable expectation of success in making the vaccine of the present invention. In the present case, the prior art provides no indication that combining only the soluble intracellular antigens with the soluble extracellular antigens wherein material having a molecular weight of less than 10,000 are removed would produce a vaccine of enhanced efficacy, i.e., a vaccine with the ability to cure horses infected for more than 60 days. At best, one skilled in the art would expect the combination to produce a vaccine that may be as efficacious as either prior art vaccine, but not a vaccine with the unexpected properties of the vaccine of the present invention. In view of Mendoza (AI), one skilled in the art may even expect that the vaccine would be less desirable, since Mendoza (AI) shows that the intracellular antigen (CMV) vaccine is less stable and more inflammatory than the extracellular antigen (SCAV) vaccine (Mendoza (AI): page 94, last paragraph). In view of Mendoza *et al.* (1996), one skilled in the art may expect an SCAV that further

included the isolated 28K, 30K, and 32K intracellular antigens to have enhanced curative properties. However, Mendoza *et al.* (1996) does not disclose what these enhanced curative properties are. Since Mendoza *et al.* (1996) teaches that the total intracellular antigen vaccines were less stable (page 161, column 1, second paragraph), one skilled in the art would not expect a vaccine that contained the total soluble intracellular antigens as claimed to have the enhanced curative properties eluded to in Mendoza *et al* (1996).

Subsequent to the holding in *In re Vaeck*, the court has consistently held that absent some teaching or suggestion that would support combining the prior art references, obviousness cannot be established by merely by combining the teachings of the prior art to make the applicant's invention. *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993), *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988), and *ACS Hospital Systems v. Montefiore Hospital*, 221 USPQ 929 (Fed. Cir. 1984). Thus, it appears that the present rejection is a hindsight reconstruction of the invention from the applicant's own disclosure, which is not permitted. As stated in *In re Vaeck*, at 1442, "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." In the present rejection, the suggestion and motivation for combining the soluble

extracellular antigens with the soluble intracellular antigens to make the vaccine of the present invention becomes obvious only in view of the applicant's disclosure, which teaches that the combination produces a vaccine with enhanced efficacy. In the absence of the applicant's disclosure, the prior art teaches that the CMV and SCAV are equivalent in efficacy, and that the SCAV is preferred over the CMV because of its longer shelf-life and its lower inflammatory reaction at the site of injection. Further, the art teaches that the most preferred vaccine consists solely of particular intracellular antigens and extracellular antigens (Mendoza *et al.* (1996), page 159, column 2, lines 15-18). There is nothing in the prior art to suggest to one skilled in the art that combining the total soluble intracellular antigens of the CMV with the total soluble extracellular antigens of the SCAV would produce a vaccine of enhanced efficacy. It is only the applicant's disclosure that teaches that a vaccine comprising both intracellular antigens and extracellular antigens produces a vaccine with the unexpected ability to cure horses infected for more than 60 days. Therefore, unless one skilled in the art had access to the applicant's disclosure, it would not have been obvious to make soluble intracellular antigens and then combine the soluble intracellular antigens with the

extracellular antigens to make the vaccine of the present invention.

Mendoza *et al.* (1996) cannot substitute for the applicant's disclosure because Mendoza *et al.* (1996) teaches a vaccine containing soluble extracellular antigens and three immunodominant intracellular antigens to provide a vaccine with some undisclosed but enhanced curative properties. It would be overly speculative to construe that the enhanced curative properties of the vaccine in Mendoza *et al.* (1996) would have led one skilled in the art to expect the vaccine of the present invention would be as "enhanced" (or more "enhanced") as the vaccine of Mendoza *et al* (1996). Therefore, because none of the prior art references can sustain an obviousness rejection either alone or in combination, it appears that the present rejection can only be sustained in view of the applicant's disclosure which is a hindsight reconstruction that is not permitted.

For the above reasons, it is believed that the prior art does not make the present invention obvious. Therefore, reconsideration of the rejection is requested.

4. The amendment filed October 17, 1999 was objected to under 35 U.S.C. § 132 because it introduces new matter.

ATCC 74446 was deposited under the Budapest

Treaty and is identical to ATCC 58643. The Budapest Treaty ATCC 74446 was identified as CBS 574.85 which is the same as ATCC 58643 which was identified as CBS 574.85 in Mendoza (AI) on page 90, column 2, line 15. Therefore, the amendment filed October 17, 1999 does not introduce any new matter into the specification.

5. Claim 21 was rejected under 35 U.S.C. § 112, first paragraph, as containing new matter.

Since ATCC 74446 deposited under the Budapest Treaty contains CBS 574.85 which is identical to the CBS 574.85 contained in ATCC 58643, no new matter has been introduced by the amendment to Claim 21 which replaces "58643" with "74446."

For the above reasons it is believed that Claims 16 to 25 are patentable. Allowance of Claims 16 to 25 is requested.

Respectfully,

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Encl. Declaration Under 37 CFR 1.132
Form 1449